Synthesis of 6-*O*-octyl-β-D-galactopyranosyl-(1→5)-L-arabinose and comparative 1-deoxyglycitolation of N^{α}-*Z*-L-lysine with amphiphilic lipodisaccharides

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A lipodisaccharide possessing a reactive aldopentose unit, 6-*O*-octyl-β-D-galactopyranosyl-(1→5)-L-arabinose **6**, was obtained by glycoside synthesis. To avoid a possible intramolecular acyl transfer, benzoyl protecting groups and mild conditions of detritylation were used in the preparation of the furanosyl acceptor. The reductive alkylation of N^{α}-*Z*-L-lysine was then studied and compared to that of previously prepared liposaccharides. In this reaction, amphiphilic five-membered hemiacetals are generally more reactive than their six-membered analogues. The newly prepared disaccharide is the most reactive of the series and also the easiest to prepare. Therefore this reagent has been selected for a future study on the chemical modification of enzymes and the use in organic solvents of the biocatalysts obtained.

Key words: liposaccharides, reductive alkylation.

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Le 6-*O*-octyl β-D-galactopyranosyl-(1→5)-L-arabinose **6**, un lipodisaccharide ayant une fonction aldopentose réactive, a été obtenu par synthèse osidique. Pour éviter un transfert d'acyle intramoléculaire dans la préparation de l'accepteur furanosyle, des groupes protecteurs benzoates et des conditions douces de détritylation ont été utilisés. Le réactif **6** a ensuite été comparé à d'autres liposaccharides dans la réaction d'alkylation réductrice de la N^{α}-*Z*-L-lysine. Dans cette condensation, les hémiacétals amphiphiles à cinq chaînons sont généralement plus réactifs que leurs analogues à six chaînons. Le lipodisaccharide **6** étant le plus réactif de la série, et aussi le plus facile à préparer, a été retenu pour une étude ultérieure de modification chimique d'enzymes.

Mots clés : liposaccharides, alkylation réductrice.

Introduction

The mild and selective reductive alkylation of the lysine residues of proteins with reducing sugars leads to interesting neoglycoproteins (1, 2). In this 1-deoxyglycitolation reaction, unsubstituted aldopentoses exhibit faster rates of condensation with amines than their more stable aldohexose analogs (3, 4). In a search for liposaccharidic reagents for the preparation of lipoglycosylated enzymes suitable for use in polar organic solvents (5), we came upon the problem of long condensation time, which could be detrimental to the catalytic activity of the new biocatalyst. For our purpose, a lipofuranose might be a valuable compound. However, in a polar solvent, a disaccharide is better able to maintain the essential layer of water around the enzyme than is a monosaccharide derivative. Therefore the synthesis of the 6-*O*-octyl-β-D-galactopyranosyl-(1→5)-L-arabinose **6**, having a reactive aldopentofuranose end, has now been achieved and its reductive condensation with N^{α}-*Z*-L-lysine compared with that of some of the previously prepared lipodisaccharides (6, 7).[2]

Results and discussion

Synthesis

The use of a stoichiometric amount of chlorotriphenylmethane in pyridine below 25°C avoids the ditritylation of the pentofuranoses and selectively gives the monotrityl derivatives (9). In these conditions, L-arabinose **1** afforded the 5-*O*-trityl-L-arabinose **2** in good yield. As benzoyl groups do not migrate as readily as acetyl ones during the cleavage of a trityl protecting group (10), benzoylation was preferred to acetylation in the next step of the synthesis. Thus, treatment of the benzoylated arabinose **3** with trimethylchlorosilane and sodium iodide (11) gave the glycosyl acceptor **4**. Königs–Knorr condensation with the previously described 2,3,4-tri-*O*-acetyl-6-*O*-octyl-α-D-galactopyranosyl bromide donor (7), in the presence of mercuric cyanide, led to the protected disaccharide **5**. Removal of the ester protecting groups yielded the liposaccharide **6**, having six free hydroxyl functions (Fig. 1).

To confirm that no intramolecular acyl transfer had occurred, the parallel synthesis of compound **6** using the very stable benzyl protection was realized. Phase transfer catalyzed alkylation of the tritylated arabinose **2** furnished the benzyl α- and β-arabinosides **3′**α and **3′**β. Cleavage of the trityl group by means of $BF_3 \cdot Et_2O$ (10) afforded the glycosyl acceptors **4′**α and **4′**β. Condensation with the same glycosyl donor as above gave the protected disaccharides **5′**α and **5′**β. Zemplén deacetylation resulted in the isolation of the benzyl *O*-(6-*O*-octyl-β-D-galactopyranosyl)-(1→5)-2,3-di-*O*-benzyl-α- and β-L-arabinosides **7**α and **7**β. Hydrogenolysis (H_2, Pd/C, MeOH) of the β-arabinoside **7**β gave a product having the same ^1H and ^{13}C NMR characteristics as those of compound **6**, unambiguously establishing the structure of that disaccharide. In an unexpected way (12), catalytic hydrogenation of the **7**α isomer under the same experimental conditions led to the alditol **8**.

Reductive alkylation of N^{α}-Z-L-*lysine*

The mechanism of the 1-deoxyglycitolation of an amine is represented in Scheme 1. The rate-determining ring opening of the hemiacetal structure *a*, to give the reactive hydroxyaldehyde *b*, depends on the stability of *a*. The effect of ring size on the stabilities of cyclic hemiacetals relative to acyclic hydroxyaldehydes has been studied in simple cases; the six-membered ring is more stable than the five-membered one, and both of them are more stable than the larger rings (13, 14). Reductive condensation of unsubstituted aldopentoses with amines is faster than that of aldohexoses, and the reductive alkylation of unsubstituted reducing disaccharides is a slow process (3, 4).

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[2]Also, D. Cabaret and M. Wakselman, manuscript in preparation.

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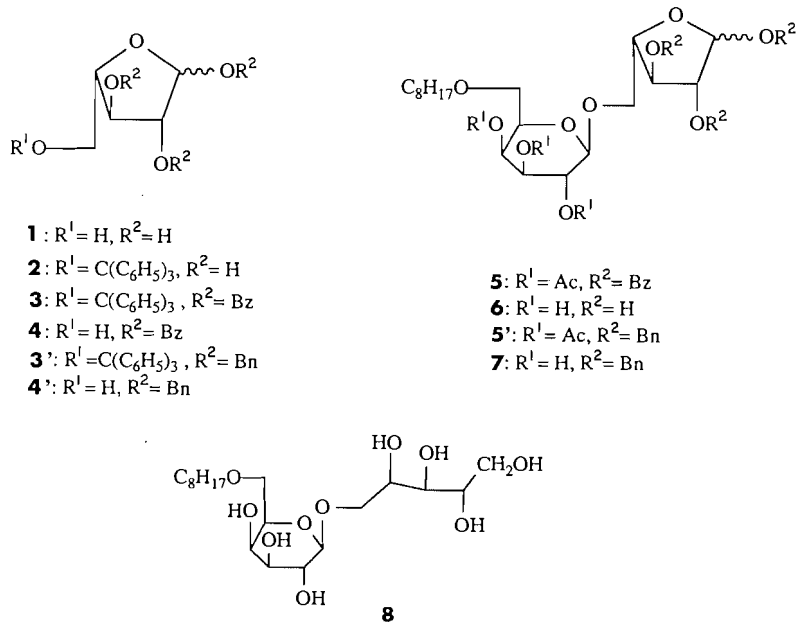
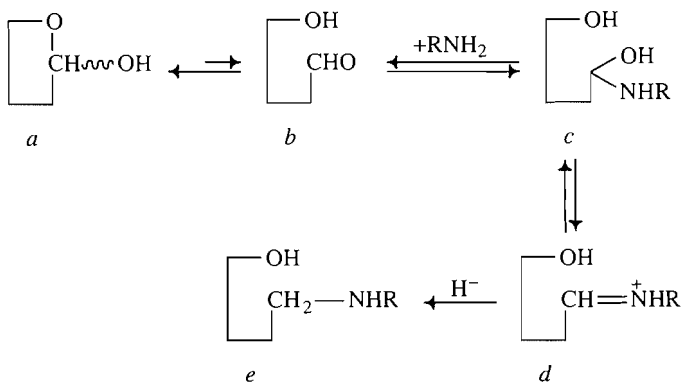


FIG. 1. Synthesis of the 6-*O*-octyl-β-D-galactopyranosyl-(1→5)-L-arabinose **6**.



SCHEME 1. Mechanism of the 1-deoxyglycitolation reaction (*a*: hemiacetal; *b*: hydroxy-aldehyde; *c*: hemiaminal; *d*: immonium ion; *e*: alkylated amine).

However, in the reactions described in the literature, an equilibrium between the furanose and the pyranose forms of the saccharide, through the acyclic hydroxyaldehyde, might occur. In the case of the 5-substituted aldopentose **6** the formation of the pyranose isomers is not possible. Furthermore, the variation of the amphiphilicity of the liposaccharide could play a role in the reaction.

A series of liposaccharides has previously been prepared (Fig. 2). We have now estimated the relative reactivities of these lipodisaccharides and compare them to that of the melibiose **9***a* and to the newly prepared reagent **6** in the model reaction with N^{α}-*Z*-L-lysine (8). Preparative condensations, under different experimental conditions, have sometimes shown the formation of small amounts of bis-deoxyglycitolated derivatives resulting from the further reductive alkylation of the monoalkylated product (see the Experimental). If we reasonably assume that the relative reactivity of the monosubstituted amine formed does not change very much by varying the nature of the disaccharide, a reactivity scale can be established by following the consumption of N^{α}-*Z*-L-lysine under mild reductive conditions close to those of the chemical modification of an enzyme (Table 1).

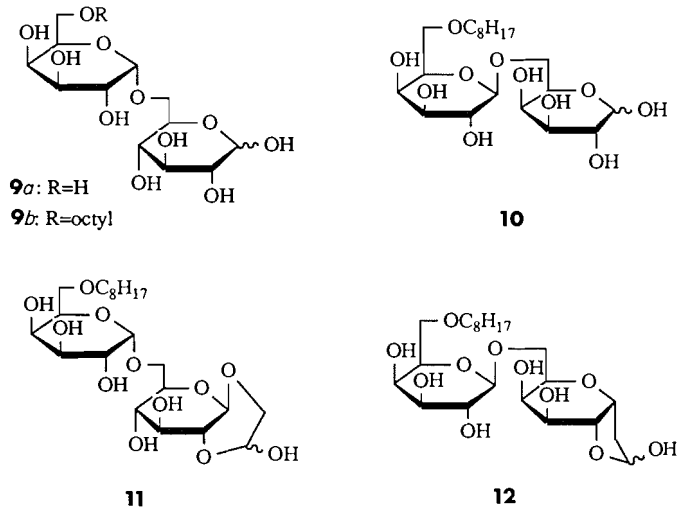


FIG. 2. Structures of the other liposaccharides: **9***b*, **10**, **11**, **12**, and melibiose **9***a*. **9***b*: 6-*O*-octyl-α-D-Gal*p*-(1→6)-D-Glc; **10**: 6-*O*-octyl-β-D-Gal*p*-(1→6)-D-Gal; **11**: 6-*O*-octyl-α-D-Gal*p*-(1→6)-β-D-gluco-pyrano[1,2-*b*]-1,4-dioxan-5-ol; **12**: 6-*O*-octyl-β-D-Gal*p*-(1→6)-α-D-galactopyrano[2,1-*b*]-oxolan-5-ol.

TABLE 1. Variation with time of the percentage of the N^{α}-*Z*-L-lysine that has reacted with the lipodisaccharides in the presence of an excess of $NaBH_3CN$ (at 4°C in a 0.1 M borate buffer)

Time (h)	Reagent					
	9*a*	**9***b*	**10**	**11**	**12**	**6**
	Percentage of consumed lysine					
4	8	9	7	20	34	30
24	13	15	12	30	52	57
48	27	31	24	39	59	66
120	31	35	30	45	63	70

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The experimental order found is the following: **6**, **12** (ref. 8) ≥ **11** (ref. 6), **10** (ref. 7), **9***b* (ref. 6), **9***a*. The lipodisaccharides having a five-membered hemiacetal structure (furanose **6** and oxolanol **12**) react more rapidly than the six-membered hemiacetals (dioxanol **11** and pyranoses **10**, **9***b*, or **9***a*). The reactivity of the 6′-*O*-octyl melibiose is not very different from that of melibiose itself. The arabinose **6** is the most reactive of the series. Moreover, its synthesis is shorter than that of the oxolanol **12**; therefore this lipodisaccharide has been selected for a future study on condensation with enzymes.

Experimental

General methods

The ^{1}H NMR spectra were recorded at 300 MHz and the ^{13}C NMR spectra at 20 or 75 MHz in $CDCl_3$ or CD_3OD. The chemical shifts (δ, in ppm) are relative to internal TMS. The optical rotations were measured with a Perkin–Elmer 241 polarimeter. The melting points were determined with a Mettler FP61 apparatus, and are uncorrected. Thin-layer chromatography was performed on precoated silica gel 60 F_{254} plates (Merck) and visualized by charring with sulfuric acid. Column chromatography was run on silica gel G60.

A Gilson HPLC chromatograph equipped with a SPD-6A Shimadzu UV spectrophotometer, a C-R 5A Shimadzu integrator, and a Brownlee C_{18} Spheri 5 (22.5 cm) column was used.

5-O-Trityl-L-arabinose **2**

The method described for the synthesis of the D(−) isomer ($[\alpha]_{589}$ +16.3° (*c* 0.85, ethanol, 20 h)) was followed (9). To a solution of L(+)-arabinose (1.5 g, 10 mmol) in 20 mL of dried pyridine was added triphenylmethane chloride (3.1 g, 11 mmol). After stirring for 24 h at room temperature, the pyridine was evaporated. The residue was dissolved in dichloromethane and washed with aqueous sodium bicarbonate. After drying over Na_2SO_4, evaporation of the solvent gave a syrup that was purified on a silica gel column (eluent: 9.1 dichloromethane–methanol) to give **2** (3.06 g, 78% yield); R_f 0.56; mp 68°C; $[\alpha]_{589}$ −15.3°, $[\alpha]_{546}$ −17.1° (*c* 0.85, ethanol, 20 h); ^{1}H NMR (CD_3OD) δ: 5.41 (H-1β, J_{1-2} = 4.5 Hz), 5.38 (H-1α, J_{1-2} = 2.5 Hz), 4.10 (H-2α and H-3α), 4.35 (H-4α), 3.45 (H-5Aα, J_{4-5A} = 3.44 Hz, J_{5A-5B} = 10 Hz), 3.38 (H-5Bα, J_{4-5B} = 5.7 Hz); ^{13}C NMR δ: 103.9 (C-1α), 97.3 (C-1β), 83.5–65.5 (C-2–C-5). Anal. calcd. for $C_{24}H_{24}O_5$: C 73.45, H 6.16; found: C 73.26, H 6.30.

1,2,3-Tri-O-benzoyl-5-O-trityl-L-arabinose **3**

To a solution of trityl arabinose **2** (1.96 g, 5 mmol) in pyridine (10 mL), benzoyl chloride (2.32 g, 16.5 mmol) was added dropwise with stirring over a period of 3 h, at room temperature. After evaporation of the pyridine, the residue was dissolved in dichloromethane and washed with a saturated sodium bicarbonate solution. Drying (Na_2SO_4) and evaporation gave a syrup that was chromatographed (85:15 pentane – ethyl acetate) to give the title compound **3** (2.9 g, 82%); R_f 0.48, the two α and β isomers were not separated; ^{1}H NMR ($CDCl_3$) δ: 6.82 (H-1β, J_{1-2} = 4.7 Hz), 6.66 (H-1α, $J_{1-2} \leq$ 1 Hz), 6.10 (H-3β, J_{3-4} = 5.6 Hz), 5.80 (H-2β, J_{2-3} = 6.6 Hz), 5.71 (H-2α, J_{2-3} = 0.9 Hz), 5.68 (H-3α, J_{3-4} = 3.3 Hz), 4.71 (H-4α), 4.5 (H-4β), 3.6 (H-5Aα), 3.55 (H-5Aβ), 3.46 (H-5Bβ), 3.40 (H-5Bα); ^{13}C NMR δ: 99.96 (C-1α), 94.77 (C-1β), 80.75–63.54 (C-2–C-5). Anal. calcd. for $C_{45}H_{36}O_8$: C 76.69, H 5.15; found: C 76.91, H 5.09.

1,2,3-Tri-O-benzoyl-L-arabinose **4**

To a solution of the trityl derivative **3** (1.6 g, 2.27 mmol) in dry acetonitrile (20 mL) under Ar at 0°C were added NaI (1.02 g, 6.8 mmol) and then chlorotrimethylsilane (0.74 g, 6.8 mmol). The mixture was stirred for 2 h at 0°C and diluted with diethyl ether. The solution was washed with water and with $Na_2S_2O_3$, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (1:1 pentane – diethyl ether) to give **4** (0.69 g, 66%); R_f 0.32; ^{1}H NMR ($CDCl_3$) δ: 6.86 (H-1β, J_{1-2} = 4.53 Hz), 6.69 (H-1α), 5.96 (H-3β, J_{3-4} = 5.6 Hz), 5.95 (H-2β, J_{2-3} = 6.68 Hz), 5.82 (H-2α, J_{2-3} = 0.9 Hz), 5.58 (H-3α, J_{3-4} = 3.7 Hz), 4.57 (H-4α), 4.38 (H-4β), 4.06 (H-5Aβ, J_{4-5A} = 4.51 Hz, J_{5A-5B} = 12.1 Hz), 4.02 (H-5Aα and H-5Bα), 3.99 (H-5Bβ, J_{4-5B} = 5.9 Hz); ^{13}C NMR δ: 99.76 (C-1α), 94.29 (C-1β), 80.76–62.23 (C-2–C-5). Anal. calcd. for $C_{26}H_{22}O_8$: C 67.53, H 4.80; found: C 67.29, H 4.80.

O-(2,3,4-Tri-O-acetyl-6-O-octyl-β-D-galactopyranosyl)-(1→5)-1,2,3-tri-O-benzoyl-L-arabinose **5**

To a solution of **4** (325 mg, 0.7 mmol) and 6-*O*-octyl-2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl bromide (**7**) (440 mg, 0.91 mmol) in dichloromethane (15 mL), was added $Hg(CN)_2$ (250 mg, 1 mmol). After stirring for 48 h at room temperature, the solution was filtered on Celite, and washed with water and a KI solution. Evaporation of the solvent gave a crude product, which was applied to a silica gel column and eluted with 7:3 pentane – ethyl acetate to give **5** (435 mg, 72%); R_f 0.54; ^{1}H NMR ($CDCl_3$) δ: 6.81 (H-1β, J_{1-2} = 4.34 Hz), 6.43 (H-1α, $J_{1-2} \leq$ 1 Hz), 5.91 (H-3β, J_{3-4} = 4.97 Hz), 5.84 (H-2β, J_{2-3} = 6.32 Hz), 5.75 (H-2α, J_{2-3} = 1.0 Hz), 5.55 (H-3α, J_{3-4} = 3.6 Hz), 5.41 (H-4′α, $J_{4'-5'}$ = 2.8 Hz), 5.31 (H-4′β, $J_{4'-5'}$ = 1.5 Hz), 5.24 (H-2′α, $J_{2'-3'}$ = 9.5 Hz), 5.16 (H-2′β, $J_{2'-3'}$ = 9.5 Hz), 5.02 (H-3′α, $J_{3'-4'}$ = 3.4 Hz), 4.94 (H-3′β, $J_{3'-4'}$ = 3 Hz), 4.60 (H-1′α, $J_{1'-2'}$ = 7.9 Hz), 4.59 (H-1′β, $J_{1'-2'}$ = 7.9 Hz), 3.83 (H-5′), 3.5–3.3 (H-6′); ^{13}C NMR δ: 101.9 (C-1′α isomer), 100.71 (C-1′β isomer), 99.94 (C-1α), 94.72 (C-1β). Anal. calcd. for $C_{46}H_{54}O_{16}$: C 64.02, H 6.31; found: C 64.21, H 6.51.

6-O-Octyl-β-D-galactopyranosyl-(1→5)-L-arabinose **6**

Compound **5** (300 mg, 0.35 mmol) was dissolved in methanol (10 mL) and treated with sodium methoxide (20 mg). After stirring for 2 h at room temperature, Amberlite CG-50 (200 mg) was added. Filtration of the solution and evaporation of the methanol gave a solid that was purified by chromatography with 8:2 dichloromethane–methanol as eluent. The title compound **6** was obtained (97 mg, 65%); R_f 0.58; mp 125°C; $[\alpha]_{589}$ −15.1°; $[\alpha]_{546}$ −18.6° (*c* 1, methanol); ^{1}H NMR (CD_3OD) δ: 5.02 (H-1β, J_{1-2} = 4.53 Hz), 4.99 (H-1α, J_{1-2} = 1.5 Hz), 4.15 (H-1′β, $J_{1'-2'}$ = 7.3 Hz), 4.13 (H-1′α, $J_{1'-2'}$ = 7.3 Hz); ^{13}C NMR δ: 105.31 (C-1′α), 105.20 (C-1′β), 103.62 (C-1α), 97.48 (C-1β). Anal. calcd. for $C_{19}H_{36}O_{10}\cdot 0.5H_2O$: C 52.64, H 8.60; found: C 52.91, H 8.55.

Benzyl 2,3-di-O-benzyl-5-O-trityl-α- and β-L-arabinosides (3′α and 3′β)

To a solution of the trityl arabinose **2** (1 g, 2.55 mmol) in benzyl bromide (3 mL) was added crushed KOH (0.5 g). After addition of tetrabutylammonium bromide (50 mg) as catalyst, the mixture was stirred overnight at room temperature. Diethyl ether was added, the ether solution was decanted, washed with water, dried (Na_2SO_4), and evaporated. The residue was chromatographed with 1:1 pentane–dichloromethane as eluent to give **3′**α and **3′**β.

3′α (670 mg, 40%); R_f 0.62; $[\alpha]_{578}$ −27.9°, $[\alpha]_{546}$ −31.7° (*c* 4, dichloromethane); ^{1}H NMR ($CDCl_3$) δ: 5.15 (H-1, J_{1-2} = 1.0 Hz), 4.27 (H-4), 4.09 (H-2, J_{2-3} = 2.8 Hz), 4.00 (H-3, J_{3-4} = 6.24 Hz), 3.29 (H-5); ^{13}C NMR δ: 105.05 (C-1), 88.25–62 (C-2–C-5).

3′β (230 mg, 14%): R_f 0.42; $[\alpha]_{578}$ +42.3°, $[\alpha]_{546}$ +47.9° (*c* 6, dichloromethane); ^{1}H NMR δ: 4.92 (H-1, J_{1-2} = 4.12 Hz), 4.22 (H-3, J_{3-4} = 5.9 Hz), 4.17 (H-4), 4.05 (H-2, J_{2-3} = 6.9 Hz), 3.29 (H-5); ^{13}C NMR δ: 98.87 (C-1), 84.27–62 (C-2–C-5).

Benzyl 2,3-di-O-benzyl-α- and β-L-arabinosides (4′α and 4′β)

To a solution of **3′**α (430 mg, 0.65 mmol) in dichloromethane (15 mL), borontrifluoride etherate (0.3 mL) and methanol (0.6 mL) were added.[2] The mixture was stirred at room temperature for 2 h. Then the reaction was poured on water, washed with saturated sodium bicarbonate, and dried (Na_2SO_4). Evaporation gave a residue, which after column chromatography (1:1 pentane – diethyl ether) yielded compound **4′**α (218 mg, 80%). **4′**α: R_f 0.36; $[\alpha]_{578}$ −82.4°, $[\alpha]_{546}$ −92.6° (*c* 2, dichloromethane); ^{1}H NMR ($CDCl_3$) δ: 5.18 (H-1, J_{1-2} = 0.97 Hz), 4.20 (H-4), 4.09 (H-2, J_{2-3} = 2.8 Hz), 4.0 (H-3, J_{3-4} = 6.2 Hz), 3.85 (H-5A, J_{4-5A} = 2.9 Hz, J_{5A-5B} = 11.9 Hz), 3.66 (H-5B, J_{4-5B} = 4.0 Hz); ^{13}C NMR δ: 105.27 (C-1), 82.89–62.26 (C-2–C-5).

The **4′**β isomer was obtained by the same method: R_f 0.28;

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$[\alpha]_{578}$ +51.3°, $[\alpha]_{546}$ +58.5° (*c* 2, dichloromethane); ^{1}H NMR δ: 4.97 (H-1, J_{1-2} = 4.43 Hz), 4.21 (H-3, J_{3-4} = 6.1 Hz), 4.07 (H-2, J_{2-3} = 7.1 Hz), 4.05 (H-4), 3.69 (H-5A, J_{4-5A} = 3.17 Hz, J_{5A-5B} = 11.9 Hz), 3.57 (H-5B, J_{4-5B} = 5.02 Hz); ^{13}C NMR δ: 99.59 (C-1), 82.33–64.03 (C-2–C-5). Anal. calcd. for $C_{26}H_{28}O_5$: C 74.26, H 6.71; found: C 74.51, H 6.70.

Benzyl O-*(2,3,4-tri-*O*-acetyl-6-*O*-octyl-β-*D*-galactopyranosyl)-(1→5)-2,3-di-*O*-benzyl-α- and β-*L*-arabinosides (5′α and 5′β)*

α Isomer

The experimental conditions were the same as those described for the synthesis of the benzoylated derivative **5** using, however, 82 mg (0.19 mmol) of the glycosyl acceptor **4′α** and 94 mg (0.19 mmol) of the 2,3,4-tri-*O*-acetyl-6-*O*-octyl-α-D-galactopyranosyl bromide glycosyl donor. Purification by chromatography (1:1 pentane-diethyl ether) yielded compound **5′α** (72 mg, 45%); R_f 0.61; $[\alpha]_{578}$ −43.4°, $[\alpha]_{546}$ −48.8° (*c* 1.3, dichloromethane); ^{1}H NMR ($CDCl_3$) δ: 5.34 (H-4′), 5.14 (H-2′, $J_{2'-3'}$ = 10.4 Hz), 4.98 (H-1, J_{1-2} = 1.4 Hz), 4.94 (H-3′, $J_{3'-4'}$ = 3.4 Hz), 4.53 (H-1′, $J_{1'-2'}$ = 8.0 Hz), 4.10 (H-4), 4.01 (H-2, J_{2-3} = 3.08 Hz), 3.90 (H-5A, J_{4-5A} = 4.3 Hz, J_{5A-5B} = 12.2 Hz), 3.88 (H-3, J_{3-4} = 6.3 Hz), 3.75 (H-5B, J_{4-5B} = 3.3 Hz), 3.73 (H-5′), 3.45 (H-6′A, $J_{5-6'A}$ = 6.0 Hz, $J_{6'A-6'B}$ = 9.8 Hz), 3.36 (H-6′B, $J_{5-6'B}$ = 6.7 Hz); ^{13}C NMR δ: 104.20 (C-1), 100.28 (C-1′), 88.50–67.85 (C-2–C-5 and C-2′–C-6′).

β isomer

Under the same experimental conditions, starting from **4′β**, **5′β** was obtained with 61% yield; R_f 0.53; $[\alpha]_{578}$ +18.4°, $[\alpha]_{546}$ +20.8° (*c* 1.1, dichloromethane); ^{1}H NMR ($CDCl_3$) δ: 5.41 (H-4′), 5.23 (H-2′, $J_{2'-3'}$ = 10.3 Hz), 4.98 (H-3′, $J_{3'-4'}$ = 3.4 Hz), 4.94 (H-1, J_{1-2} = 4.3 Hz), 4.50 (H-3), 4.47 (H-1′, $J_{1'-2'}$ = 7.8 Hz), 4.10 (H-4), 4.06 (H-2, J_{2-3} = 7 Hz), 3.96 (H-5A, J_{4-5A} = 6.4 Hz, J_{5A-5B} = 10 Hz), 3.79 (H-5′), 3.67 (H-5B, J_{4-5B} = 6.1 Hz), 3.50 (H-6′A, $J_{5-6'A}$ = 5.9 Hz, $J_{6'A-6'B}$ = 9.7 Hz), 3.42 (H-6′B, $J_{5-6'B}$ = 6.8 Hz); ^{13}C NMR δ: 101.02 (C-1′), 99.07 (C-1), 84.47–67.74 (C-2–C-5 and C-2′–C-6′). Anal. calcd. for $C_{46}H_{60}O_{13}$: C 67.29, H 7.37; found: C 67.20, H 7.37.

Benzyl O-*(6-*O*-octyl-β-*D*-galactopyranosyl)-(1→5)-2,3-di-*O*-benzyl-α- and β-*L*-arabinoside (7α and 7β)*

Compounds **5′α** and **5′β** were deacetylated by the same procedure used for compound **5**. The title compounds were purified by column chromatography (9:1 dichloromethane–methanol) (80% yields).

α Isomer: R_f 0.68; $[\alpha]_{578}$ −58.5°, $[\alpha]_{546}$ −65.2° (*c* 1.1, dichloromethane); ^{1}H NMR ($CDCl_3$) δ: 5.28 (H-1, J_{1-2} = 0.9 Hz), 4.27 (H-1′, $J_{1'-2'}$ = 6.8 Hz), 4.21 (H-4), 4.06 (H-2), 4.05 (H-5A, J_{4-5A} = 2.7 Hz, J_{5A-5B} = 12.7 Hz), 3.94 (H-3), 3.82 (H-5B, J_{4-5B} = 2.3 Hz), 3.73 (H-6′A, $J_{5'-6'A}$ = 5.4 Hz, $J_{6'A-6'B}$ = 10.1 Hz), 3.66 (H-6′B, $J_{5'-6'B}$ = 5.2 Hz); ^{13}C NMR δ: 104.95 and 105.45 (C-1 and C-1′).

β Isomer: R_f 0.62; $[\alpha]_{578}$ +12.2°, $[\alpha]_{546}$ +13° (*c* 0.7, dichloromethane); ^{1}H NMR δ: 4.98 (H-1, J_{1-2} = 4.3 Hz), 4.24 (H-1′, $J_{1'-2'}$ = 7.6 Hz), 4.23 (H-3, J_{3-4} = 6 Hz), 4.12 (H-4), 4.05 (H-2, J_{2-3} = 6.7 Hz), 4.04 (H-2′, $J_{2'-3'}$ = 9.3 Hz), 3.97 (H-3′, $J_{3'-4'}$ = 3.1 Hz), 3.72 (H-5A, J_{4-5A} = 5.6 Hz, J_{5A-5B} = 10.2 Hz), 3.64 (H-5B, J_{4-5B} = 7 Hz), 3.44 (H-5′); ^{13}C NMR δ: 103.35 (C-1′), 99.29 (C-1).

Hydrogenolysis of 7α

To a solution of compound **7α** (36 mg, 0.05 mmol) in methanol (15 mL), 30 mg of 5% Pd/C was added, and the hydrogenolysis was performed in a Parr apparatus for 2 h under a 50 psi hydrogen atmosphere (1 psi = 6.9 kPa). The catalyst was removed and the filtrate concentrated. Chromatography (7:3 ethyl acetate – methanol) yielded **8** (18 mg, 80%); R_f 0.28; ^{1}H NMR (CD_3OD) δ: 4.46 (H-1′, $J_{1'-2'}$ = 7.3 Hz), 3.75 (H-2′, $J_{2'-3'}$ = 9.7 Hz), 3.68 (H-3′, $J_{3'-4'}$ = 3.1 Hz); ^{13}C NMR δ: 105.32 (C-1′), 75.05–64.90 (C-2–C-5 and C-2′–C-6′). The ^{1}H and ^{13}C NMR spectra are identical to those of the reference product obtained by reduction of **6** with $NaBH_3CN$.

Hydrogenolysis of 7β

Under the same experimental conditions, compound **7β** (15 mg) was hydrogenolysed in the presence of Pd/C (35 mg) to give **6**. A second hydrogenolysis did not give the reduced product **8**. Thin-layer chromatography showed, however, the presence of a small amount of **8** in addition to **6**.

Determination of the N^{α}*-Z-L-lysine 1-deoxyglycitolation reactivity scale*

The reactions with the various disaccharides were performed simultaneously. In a typical experiment, N^{α}-Z-L-lysine (5.62 mg, 0.02 mmol) was dissolved in 1 mL of a 0.1 M borate buffer, pH 8.0. The mixture was cooled to 4°C, then 0.05 mmol of the carbohydrate was added. Stirring was maintained for 30 min before addition of $NaBH_3CN$ (32 mg, 0.5 mmol). Aliquots (50 μL) were withdrawn at intervals, directly diluted with 4 mL of the HPLC eluent (acetonitrile–water: 35–65 v/v), and 5 μL of the resulting solution was injected. The reaction was followed by comparing the initial and the sample absorbance of the N^{α}-Z-L-lysine HPLC peak at 190 nm. The values obtained were reproducible in a 3% range.

Preparative coupling of liposaccharides with N^{α}*-Z-L-lysine*

Under different experimental conditions, preparative non-optimized experiments were performed to characterize the end products.

Reductive alkylation with 6

Carbohydrate **6** (42 mg, 0.1 mmol), N^{α}-Z-lysine (112 mg, 0.4 mmol), and sodium cyanoborohydride (63 mg, 1 mmol) were dissolved in a 0.1 M borate buffer, pH 8.0 (2 mL). Stirring at room temperature was maintained for 16 h. The mixture was evaporated to dryness and the residue chromatographed on silica gel, and eluted with methanol to give reduced carbohydrate **8** (11 mg, 25%) and 1-deoxyglycitolated N^{α}-Z-L-lysine (36 mg, 52%); R_f 0.38; ^{1}H NMR (CD_3OD) δ: 0.9 (3H, octyl), 5.1 (2H, *Z*), 7.2 (5H, *Z*). High resolution MS (FAB^+), calcd. for MH^+, $C_{33}H_{57}N_2O_{13}$: 689.386; found: 689.431.

*Reductive alkylation with 9*b

A solution of N^{α}-Z-L-lysine (45 mg, 0.16 mmol), ***9b*** (75 mg, 0.16 mmol), and sodium cyanoborohydride (100 mg) in water (5 mL) was adjusted to pH 7 with 0.1 M hydrochloric acid. The solution was kept for 3 days at 60°C. Water was evaporated and the product was purified by chromatography on silica gel, with water as eluent. ***9b*** (18 mg, 25%), reduced carbohydrate (15 mg, 20%), and alkylated amine (34 mg, 30%) were isolated; R_f 0.43 (water–methanol – ethyl acetate, 1:2:3); ^{1}H NMR (CD_3OD) δ: 0.85–1.80 (15H octyl + 6H lysine), 3.0–4.5 (massif), 5.06 (2H benzyl), 7.2–7.4 (5H benzyl). High resolution MS (FAB^+), calcd. for MH^+, $C_{34}H_{59}N_2O_{14}$: 719.396; found: 719.352.

Reductive alkylation with 10

Using the same experimental conditions as for ***9b***, the 1-deoxyglycitolated N^{α}-Z-L-lysine was obtained in 22% yield; R_f 0.48 (water–methanol – ethyl acetate: 1:2:3); ^{1}H NMR (CD_3OD) δ: 0.8–0.95 (3H, octyl), 1.20–1.40 (12H, octyl), 1.40–1.80 (6H, lysine), 2.90–3.20 (2H, lysine and 2H on C1), 3.40–4.20 (H lysine and carbohydrate), 4.28 (d, *J* = 8, H1′), 5.05 (2H, benzyl) and 7.15–7.25 (5H, benzyl). High resolution MS (FAB^+), calcd. for MH^+, $C_{34}H_{59}N_2O_{14}$: 719.396; found: 719.367.

Reductive alkylation with 12

Compound **12** (50 mg, 0.104 mmol), N^{α}-Z-L-lysine (29 mg, 0.104 mmol), and sodium cyanoborohydride (65 mg, 1.04 mmol) in 0.1 M borate buffer, pH 8 (5 mL), were stirred for 16 h at room temperature. After chromatography, 39 mg of a mixture of mono- and di-alkylated amine (in a 72:28 ratio) was isolated; R_f 0.39 (methanol). High resolution MS (FAB^+): monoalkyl, calcd. for MH^+, $C_{36}H_{61}N_2O_{14}$: 745.412; found: 745.400; and dialkyl, calcd. for MH^+, $C_{58}H_{101}N_2O_{24}$: 1209.6; found: 1209.4.

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